**Dogo Rangsang Research Journal** **UGC Care Group I Journal**
**ISSN : 2347-7180** **Vol-08 Issue-14 No. 04: 2021**

# A perspective on under water photo synthesis in sub merged terrestrial wetland plants

[1]RACHITA RAUL, *Gandhi Institute of Excellent Technocrats, Bhubaneswar, India*
[2]RAJ KISHORE JENA, *Mahavir Institute of Engineering and Technology, Bhubaneswar, Odisha, India*

## Abstract

Background andaims

Wetland plants inhabit flood-prone areas and therefore can experience episodes of completesubmergence.Submergenceimpedesexchangeof$O_2$and$CO_2$betweenleavesandtheenviron-ment, and light availability is also reduced. The present review examines limitations to under-waternetphotosynthesis($P_N$)byterrestrial(i.e.usuallyemergent)wetlandplants,ascomparedwithsubmergedaquaticplants,withfocusonleaftraitsforenhanced$CO_2$acquisition.

Scope

Floodwatersarevariableindissolved$O_2$,$CO_2$,lightandtemperature,andtheseparametersinfluenceunderwater$P_N$andthegrowthandsurvivalofsubmergedplants.Aquaticspeciespossess morphological and anatomical leaf traits that reduce diffusion limitations to $CO_2$uptake and thus aid $P_N$ under water. Many aquatic plants also have carbon-concentratingmechanisms to increase $CO_2$ at Rubisco. Terrestrial wetland plants generally lack the numer-ous beneficial leaf traits possessed by aquatic plants, so submergence markedly reduces $P_N$.Some terrestrial species, however, produce new leaves with a thinner cuticle and higher spe-cificleafarea,whereasothershaveleaveswithhydrophobicsurfacessothatgasfilmsareretained whensubmerged;bothimprove$CO_2$entry.

Conclusions

Submergence inhibits $P_N$ by terrestrial wetland plants, but less so in species that produce newleaves under water or in those with leaf gas films. Leaves with a thinner cuticle, or those withgasfilms,haveimprovedgasdiffusionwithfloodwaters,sothatunderwater$P_N$isenhanced.Underwater$P_N$providessugarsand$O_2$tosubmergedplants.Floodwatersoftencontaindis-solved $CO_2$ above levels in equilibrium with air, enabling at least some $P_N$ by terrestrialspecieswhensubmerged,althoughratesremainwellbelowthoseinair.

## Introduction

Emergentwetlandplantsarewelladaptedtowater-logged soils, but can also experience episodes of com-pletesubmergence.Completesubmergencehas an impact on wild species in coastal marshes and riverfloodplains(Armstrong*etal*.1985),and many ricecropsaregrowninregionsthreatenedby floods,causingsubmergence(JacksonandRam2003).Complete submergence impedes the exchange of $O_2$and$CO_2$betweenleavesandtheenvironment(MommerandVisser2005;Voesenek*etal*.2006).Lightavailabilityto submerged plants also decreases, and markedly sowhen floodwaters are turbid (Mommer and Visser 2005;Voesenek *et al*. 2006). Restricted photosynthesis, butongoing substrate consumption in respiration or fermen-tation, causes sugars to become depleted in submergedplants, which in turn can result in damage or even deathfrom substrate exhaustion (Bailey-Serres and Voesenek2008;ColmerandVoesenek2009).

Theinterfacebetweenlandandwaterisnotwelldefinedas watertablesfluctuatewithprecipitationandevaporation,sothatplantsexperiencevariableperiodsanddepthsofflooding(Sculthorpe1967).Plantsexploitnichesacrossthesedynamicfloodinggradients, butfunctionalclassificationofplanttypeslackssharpboundariesowingtothecontinuumofdiversity.Notwithstandingthesedifficulties,plantsfromthewettestendofthegradienthavebeenclassifiedintotwomaingroups:(i)aquaticplantsthatprimarilylivecompletelysubmergedand(ii)amphibiousplantsthatlivewithemergentshootsordevelopwaterformswhensubmerged(Iversen1936;citedbySculthorpe1967).Emergentwetlandplantstypicallymaintainalargeportionoftheirshootsinair,butoccasionallybecomecompletelysubmerged.Toclearlydistinguishtheseemergentplantsfromotherwetlandspecieswithshootportionsinair(e.g.emergentamphibiousplants),werefertothisfunctionalgroupas‘terrestrialwetlandplants’(presentreview; ColmerandPedersen2008;Pedersen*etal*.2010).Suchdistinctionisimportantasterrestrialwetland plants typically grow vigorously in waterloggedsoilsand/orareaswithshallowstandingwater,withthedepthlimitbeingdeterminedbycapacityfortransportofatmospheric$O_2$tobelowgroundtissues(Sorrell*etal*.2000).Forsubmergedterrestrialplants,$O_2$deficiencyandescape responses via shoot elongation have been elucidated,revealingsophisticatedsignalling,changesingeneexpressionandalteredmetabolismduringsubmergence(e.g.reviewedbyBailey-SerresandVoesenek2008,2010).Thecapacityforsomenetphotosynthesis($P_N$) to continue when under water enhances plant toleranceofsubmergence,as$P_N$provides$O_2$forinternalaerationandsugarsforenergymetabolismandgrowth (MommerandVisser2005).

Thepresentreviewexamineslimitationstounderwater$P_N$by terrestrialwetlandplantsand comparestheir functioning with aquatic plants. Our focus here onunderwater$P_N$asrelatedtotheecophysiologyofsubmergence tolerance adds to the vast knowledge on root adaptations in wetland species. Roots of wetlandplants typically contain large volumes of aerenchyma,oftenabarriertoradial$O_2$loss,andtheabilityto tolerate tissue $O_2$ deficits and reduced phytotoxins inwaterlogged soils (Armstrong 1979; Jackson and Armstrong 1999; Bailey-Serres and Voesenek 2008; Colmerand Voesenek 2009). Here, we show that underwater$P_N$bysubmergedterrestrialwetlandplantsislimitedby $CO_2$ availability even though floodwaters commonlycontain dissolved $CO_2$ above air equilibrium, and so leaftraitsinfluencingunderwater$P_N$areimportantforsubmergencetolerance.

## Thesubmergenceenvironmentduringoverlandfloods

Floodsdifferin seasonaltiming,duration, depthandfrequency(e.g.Vervuren*etal*.2003).Floodwaterproperties (e.g. water turbidity and dissolved $CO_2$) that influenceplantfunctioningcan also differ substantially;light and $CO_2$ available to submerged plants determineunderwater $P_N$ and survival (Mommer and Visser 2005;Pedersen *et al*. 2010). Thus, the flooding regime andwater properties influence plant species distributions inflood-proneareas(Armstrong *et al*. 1985; Voesenek*et al*. 2004). In this section, we discuss three types offloodingeventsthatcanaffect terrestrial wetlandplants:flashfloods,seasonalfloodsandtidalflooding.

Flash floods occur when heavy rainfall causes waterlevels to rise rapidly for a variable period of time, especially as run-off moves to low-lying areas (Setter *et al*.1987; Brammer 1990; Ram *et al*. 1999). Flash floods insome regions can be more likely to occur during specificseasons, but in other areas flash flooding is not seasonspecific. Seasonal floods are caused by an increase inwaterflowthatsurpassesthecapacityofriversinalandscape to discharge the large volumes of water, resultingin overflow of banks and floodplains. The origin of theincreasedwaterflowcanbeseasonalprecipitationand/orsnowmelt(Brammer1990).Tidalfloodingimpactscoastalplainsandestuarinemarsheswithdepthsdeterminedbythemoon’s cycle (e.g. neaptidesandspringtides).Tidalfloods involve salinewater, whereas overland floods are usually freshwater,withtheexception of some inland catchments withsaltlakes.

Floodingcanoccurwithvariouscombinationsofchemical and physical properties in the water; $O_2$, $CO_2$,temperature,pHandlightcanallvary(Setter*et al*.1987;Pérez-Llorens*etal*.2004).SeawaterpHiswellbufferedasitcontains$HCO_3^-$(2.2mM;Millero*etal*.1998)and$HCO_3^-$alsobuffersagainstseveredepletionofdissolved$CO_2$.Infreshwaterfloods,$HCO_3^-$and$CO_2$ concentrationsarehighlyvariable,butdissolved$CO_2$iscommonly above air equilibrium (Table 1). The high $CO_2$concentrationstypicallyresultfromrespirationby

Table1Dissolved$CO_2$and$O_2$concentrationsinvarioustypesoffloodwaters.Medianswithrangesinparentheses.

| Environment | $CO_2$(mM) | $O_2$(mM) |
|---|---|---|
| Terrestrial | | |
| Flashflood(1,2)(*n*¼4) | 1040(3–1953) | 150(‘0’–280) |
| Seasonalflood(326) (*n*¼6) | 365(47–1600) | 79(‘0’–240) |
| Tidalflood(7,8)(*n*¼4) | 16(3–49) | 292(188–522) |
| Aquatic | | |
| Streamsandrivers(9,10) (*n*¼31) | 133(11–836) | n.a. |
| Ponds(<1ha)(11) (*n*¼7) | 59(<1–374) | n.a. |
| Lakes (11)(*n*¼17) | 45(11–210) | n.a. |

[1] (Ram*etal*.1999;Setter*etal*.1987);[326](Hamilton*etal*.1997; H miltonetal.1995;Richey*etal*.2002;Valett*etal*.2005);
[7] (Pérez-Lloréns*etal*.2004;Winkel*etal*.2011);[9](Sand-Jensenandrost-FChristensen1998);[10](Jonsson*etal*.2003)[11](Staehr*etal*.2011).a.,notavail nable.
C $_2$wasnotmeasuredinthewatersurveysconductedin9,10and11.

organisms consuming labile carbon compounds (i.e. anetheterotrophicsystem);inaddition,somewaterbodies receive $CO_2$-enriched groundwater stream flows.By contrast, in net autotrophic systems photosynthesisdepletes$CO_2$andproduces$O_2$.So,$O_2$concentrationsinfloodwaterscanrangefromseverelyhypoxic(net heterotrophic)towellaboveairequilibrium(netautotrophic).

Temperatureduringfloodingeventscanalsovarywidely(e.g.~6–378C;Hamilton*etal*.1997;Valett *et al*. 2005; Pedersen *et al*. 2011*a*), depending on location and season. Respiration increases at warmer temperatures,whichcandeplete$O_2$,and$O_2$concentrationisfurtherreducedowingtolower$O_2$solubilityinwateras temperature increases. So, the imbalance between$O_2$ demand and supply to submerged terrestrial plantscanbefurtherexacerbatedastemperatureincreases.

Flow rates during floods have only been reported, toour knowledge, in three papers: data are available fortwoflashfloodsandoneseasonal river flood, andflowsrangedfrom0.002to0.3ms$^{21}$.Flowratesaffectthe thickness of diffusive boundary layers (DBLs) andthereby influence gas and nutrient exchanges with submergedplants(Binzer*etal*.2005;Pedersen*etal*.2009).So,underwater$P_N$canincreasewithincreasingflowvelocitysincetheDBLsbecomethinner(Jones*etal*.2000),buttheresponsewouldplateau(cf.$O_2$supply;Binzer*et al*. 2005) or even decline again if flows cause excessiveshootagitation(Madsen*etal*.1993*a*).

Lightregimesinfloodwatersaredependentonseveralfactors. When floodwaters contain suspended particlesor dissolved coloured organic matter (e.g. tannins inAmazonian floodwaters; Parolin 2009), light availabilitywill be reduced. Particle suspension can be highestduring early stages of floods and particles often thensettle; however, if particles settle on leaves these canstill limit light. Waters of high nutrient availability typic-ally support growth of microalgae, with dense populations of both biofilms and phytoplankton leading tolower light penetration to leaf surfaces (Sand-Jensenand Sondergaard 1981; Sand-Jensen and Borum 1991;Lassen *et al*. 1997) and consequently also shallowerdepth limits for plant colonization (Sand-Jensen 1990).Examples of light reductions are available for flood-waters in the rice fields of India and Thailand; thedepthatwhich50%lightremainedvariedfrom0.07to 0.7m(Setter*etal*.1987;Ram*etal*.1999).

Howdoesthesubmergedenvironmentexperiencedbyterrestrialwetlandplantscomparewiththatofwaterbodiescontainingpermanentaquaticvegetation?Inbrief, environments supporting healthy stands of sub-merged aquatic plants, such as the shallow sea, andareas within rivers and lakes, also share many of theabove-mentionedconstraintstoplantgrowth.Lightattenuation in the water column (caused by water itself,dissolved coloured organic matter, phytoplankton andotherparticles)determinesthemaximumdepthofcolonization by aquatic plants. Seagrasses typically grow downto ~10% of the surface light (Duarte 1991), whereas thedepth penetration of plants in freshwater lakes is downto<1%andtypically~5%ofthesurfacelight(Canfield *et al*. 1985). The lower light compensation points for thegrowth of deep– colonizing freshwater plants result fromthesehavinghighershoot-to-rootratiosthanseagrasses.The deepest-growing freshwater plants, such as species of*Ceratophyllum*and*Utricularia*,donotproducerootsatall(Cook 1990). Similar to terrestrial floodwaters, dissolvedinorganiccarbon(DIC)infreshwatercanalsovary widely(e.g. from 0.02 to 5.6 mM in British lakes; Maberly andSpence1983).DependingonpH,theaboveDICconcentrationsmayresultindissolved$CO_2$levels fromnearorbelow airequilibrium(15mMinfreshwaterat208C)towatersin streams/rivers,pondsandlakesthataretypicallysupersaturated(Table1);pondscanevencontainupto2000mM$CO_2$ (133-fold air equilibrium). The temperature in mostwater bodies fluctuates significantly less than surround-ing air due to the much higher specific heat capacity ofwater compared with air (Hutchinson 1957), but thereare exceptions, such as in shallow rock pools with largedielfluctuations(Pedersen*etal*.2011*a*).Finally,theflowvelocity in aquatic environments also varies widely, asdescribed earlier for terrestrial floods, from almost stagnantconditionsinpondsanddeeperareasoflakestovery high velocities in rivers and in surf zones of the sea(2–3ms$^{21}$;Vogel1994).Infast-flowingwaterorinwave-zones, the strap-shaped leaves typical of some aquaticplants are highly adaptive as this morphology reducesthepressuredrag(Vogel1994).

In summary, floodwaters faced by terrestrial plantsinvokesomecommonconstraintsofrestrictedgasexchangeandlowerlightavailability, but conditions($O_2$,$CO_2$,lightandtemperature)differbetweenlocationsand times, posing variable challenges to plant functioningduringsubmergence.Floodwaterchemicalandphysical properties, in addition to the well-recognizedimportance of seasonal timing, duration, depth and frequencyoffloods(e.g.Vervuren*etal*.2003),willinfluenceplant growthandsurvivalduringsubmergence.

## Netphotosynthesisunderwater

Low $CO_2$ and/or low light can restrict $P_N$ by submergedplants (Sand-Jensen 1989). This review focuses on $CO_2$acquisition.Aquaticspeciespossessleaftraitstoenhance DIC supply and thus ratesof underwater $P_N$.InTable2,wecomparetheleaftraitsofterrestrialwetlandplantswith those of submerged aquaticplants. Below we (i) summarize knowledge of morphologicalandanatomicalleaftraits,andphotosyntheticpathwaysincludingcarbon-concentratingmechanisms(CCMs), and (ii) compare the rates of underwater $P_N$ bydifferenttypesof aquatic and terrestrial wetlandplants,asinfluencedbytheseleaftraits.

## Leaf traits of terrestrial wetland plantsandsubmergedaquaticplants

Leaf morphology determines boundary layer resistancesto exchange of dissolved gases and ions (Madsen andSand-Jensen 1991). Boundary layer resistance can limittheratesof$CO_2$uptakeandthusreduceunderwater$P_N$in submergedplantsasdiffusionis$10^4$-foldslowerin water than in air (Vogel 1994). Morphological traits(Table 2) that reduce the DBL resistance, by decreasingthedistancetothe 'leading edge' (Vogel 1994),includeleafshapesofsmall,dissected/lobedand/orstrap-likeleaves.Inaddition,aquaticleaveslacktrichomes,thusavoidingthedevelopmentofthickerboundary layers adjacent to their surfaces. Leaves ofaquatic species also tend to be thin (Table 2), althoughthereareseveralexceptions(e.g.isoetids;Sand-JensenandPrahl1982).Thinleaveshaveshortinternaldiffusion

Table 2 Comparison of leaf traits influencing gas exchangeandphotosynthesisbyterrestrialwetlandplantswhen underwater and by submerged aquatic plants. Modified fromSculthorpe (1967) with data from additional references asindicatedby superscripts:[1](NeinhuisandBarthlott1997), [2](ColmerandPedersen2008),[3](MaberlyandMadsen2002).

| Leaftraits | Terrestrial wetlandplants | Submerged aquaticplants |
|---|---|---|
| Morphology | | |
| Leafsize | Medium–large | Small–medium |
| Dissected/lobed | Rare | Common |
| Strap-shaped | Rare | Common |
| Leafthickness[a] | Moderate–thick | Thin |
| Surface hydrophobicity/leaf gasfilms[1,2] | Common | Absent |
| Hairs/trichomes | Rare | Absent |
| Anatomy | | |
| Stomata | Alwayspresent | Absent/ non-functional |
| Cuticle | Alwayspresent | Absent/highlyreduced |
| Chloroplastsin epidermalcells | Onlyinguard cells | Common |
| Aerenchyma | Variable | Variable |
| Porosity oflamina | Highinthick, lowinthin, lamina | Highinthick,lowin thin, lamina |

1982).Incaseswhereleavesarerelativelythick,$CO_2$istypicallysourcedfromsediments(e.g.isoetids;Winkel
path lengths, reducing the overall resistance for $CO_2$ toreachchloroplasts(MadsenandSand-Jensen1991;Maberly and Madsen 2002). One example is the laminaof*Najasflexilis*,whichisonlytwocelllayers(Tomlinson andBorum2009),andtheseleavestendtobeofhighporositytofacilitateinternalgasphasediffusion(Pedersen and Sand-Jensen 1992; Pedersen *et al*. 1995;Sand-Jensen*etal*.2005).

Inadditiontothesemorphologicaltraits,leavesofaquatic species also have anatomical traits that furtherreducediffusiveresistancesforCO$_2$toreachchloroplasts(Table2).Aquaticleaveslack,orhaveveryreduced,cuticles. Diffusion across the cuticle is the main pathway ofdissolvedgasexchangeastheleaveslackstomata,orifpresent, the stomata are non-functional (Pedersen andSand-Jensen 1992). The diffusion path length to chloroplastsisalsominimizedbyhavingtheseorganellesinallepidermal cells, and in sub-epidermal cells the chloroplastsarepositionedtowardstheexterior(Table2).

Submerged aquatic plants also display physiologicaladaptationstoincreasetheCO$_2$concentrationatRubisco,the site of carboxylation (Table 2)—these are referred toasCCMs(MaberlyandMadsen2002;Raven*et al*.2008).In submergedaquaticplants,CCMsincludeHCO$_3^-$use (Prins and Elzenga 1989), C4 (Magnin *et al*. 1997), C3–C4intermediates(Keeley1999)andCrassulaceanAcidMetabolism(CAM)photosynthesis(Keeley1998).Carbon-concentrating mechanisms increase $P_N$ in CO$_2$-limitedsubmergedenvironments,andhavealsobeensuggestedtodiminishphotorespiration(MaberlyandMadsen2002).PhotorespirationresultsfromtheoxygenaseactivityofRubiscoandispromotedbyalowCO$_2$:O$_2$ratio(Ogren1984), a condition common in leaves when under water(Bowes1987).ThelowCO$_2$availabilityinaquaticenviron-mentswouldinitselflowertheCO$_2$:O$_2$ratio.Moreover,O$_2$in submerged leaves can be high as escape is slower thanproduction in $P_N$; O$_2$ escape is not only hampered by DBLsbutalsobytherelativelylowO$_2$solublityinwater;CO$_2$is 28.5-foldmoresolublethanO$_2$at20°C(Baranenko*etal*. 1990).ReducedphotorespirationinasubmergedaquaticCAM plant has been recently demonstrated (Pedersen*et al*. 2011*b*), supporting the view that CCMs do reducephotorespirationinaquaticspecies.

By contrast with aquatic species, leaves of terrestrialwetlandplantslackmostofthefeatures describedabove (Table 2) and so suffer from large diffusion limitationstoCO$_2$supplyfor$P_N$whenunderwater,unlesstheypossess leaf gas films (Raskin and Kende 1983; Colmerand Pedersen 2008; Pedersen *et al*. 2009) or producesubmergence-acclimated leaves (Mommer and Visser2005).Below,we evaluate underwater $P_N$ by leavesofterrestrialwetlandplantsandthenconsidertheoccurrenceandfunctioningofleafgasfilms.

## Netphotosynthesisofaquaticandsubmergedterrestrialwetlandplants;leaftraitsenhanceCO$_2$supply

The most comprehensive comparison of underwater $P_N$by aquaticandterrestrial wetland plants is the studyby Sand-Jensen *et al*. (1992). Thirty-five species of fourlifeforms(terrestrial,amphibioushomophyllous, amphibious heterophyllous and aquatic species) werecompared (listed in Appendix 1). Inclusion of data fromotherstudiesinthepresentanalysiswasconstrainedby differencesintechniquesand conditionsusedforunderwater $P_N$ measurements, e.g. CO$_2$, temperatureandlight(Appendix2).

Classificationsofwetlandplants into functionalgroups are convenient, but are also imperfect as theboundaries are not sharp (see Introduction). As examples,someterrestrialwetlandspeciesproducenewleaves when submerged and these can display someacclimationtothe underwater environment (Mommer*et al*. 2007). Similarly, homophyllous amphibious plantscan also display some acclimation, e.g. thinner cuticlesand modestly thinner leaves when formed under water(Nielsen 1993), but these changes are far more subtlethanthosedisplayedbyheterophyllousamphibiousplants. Not surprisingly, different authors have classifiedsomespeciesintodifferentlifeforms.Here,ourfocusisonthecomparisonofunderwater$P_N$ of leavesformed(i)inairbyterrestrialwetlandspecies, (ii) underwaterbyamphibioushomophyllousspecies, (iii) under water by amphibious heterophyllous speciesand(iv)underwaterbyaquaticspecies.

An additional noteworthy feature of the study bySand-Jensen *et al*. (1992) was documentation of dis-solved CO$_2$ levels in lowland stream habitats. Under-water $P_N$ was measured at ambient and at elevatedCO$_2$ concentrations, to provide rates of relevance tothe field situation as well as CO$_2$-saturated $P_N$ foraquatic leaf types. The level of elevated CO$_2$ used(~800 mM, being ~50-fold air equilibrium) would havesaturated $P_N$ by the aquatic leaf types. It is uncertainwhether rates were CO$_2$ saturated for some of theterrestrial leaf types, which can require as much as75-fold of air equilibrium CO$_2$ when submerged (ColmerandPedersen2008).

We compare the rates on the dry mass basis (Fig. 1Aand B) used by Sand-Jensen *et al*. (1992) and also on aprojected leaf surface area basis (Fig. 1C and D); conversions used specific leaf area (SLA) data in the literature(Fig.2;Appendix1).DataforSLAwerenotavailableforthree oftheaquaticandthreeoftheter-restrial wetland species in Sand-Jensen *et al*. (1992), sothesesixwereomittedfromthepresentanalysis(Appendix1).

Theoverallbeneficialeffectsofaquaticleaftraits(Table 2) for underwater $P_N$, as well as the generallypoor performance of leaves of terrestrial plants, wereclearlydemonstratedinSand-Jensen*etal*.(1992).These authors highlighted that (i) underwater $P_N$ on amass basis increased from terrestrial, then amphibious,totrulyaquaticleaftypesand(ii)Danishlowland

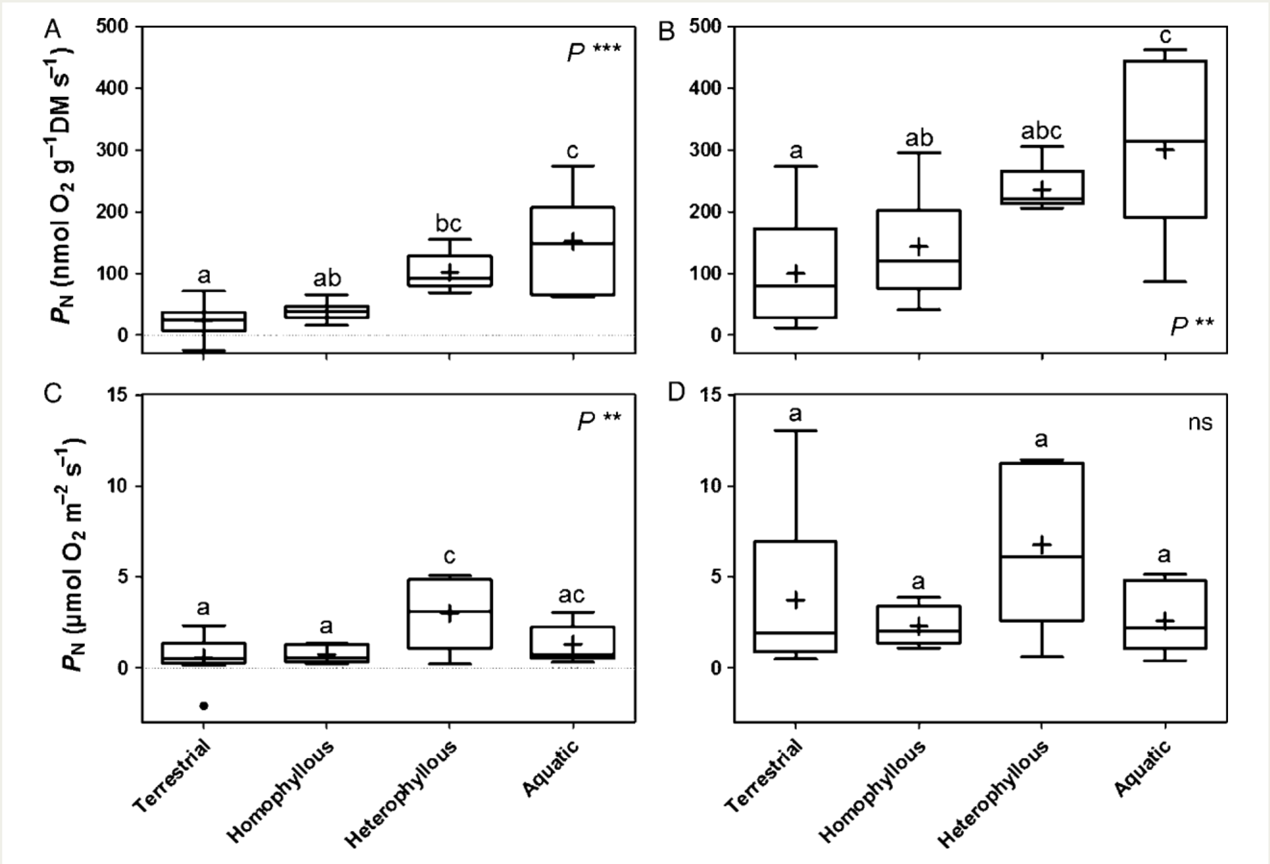

Fig.1Underwaternetphotosynthesis($P_N$)interrestrialwetlandplants,inamphibioushomophyllousorheterophyllouswetland plantsandinsubmergedaquaticplants.Netphotosynthesiswasmeasuredat158Candisexpressedperleafdrymass(AandB)or perprojectedleafarea(CandD)atambient$CO_2$levels(90–400mMinthenaturalhabitats;AandC)oratelevated$CO_2$levels(800 mM; B and D). Species and SLA data sources are listed in Appendix 1. Our analysis focused on the study by Sand-Jensen *et al*.(1992) as it is the most comprehensive available; addition of other data was constrained by differences in techniques and conditionsused(e.g.$CO_2$andtemperature;Appendix2).Terrestrial,leavesformedinairbyemergentwetlandplants;homophyllous,leavesformed underwaterbyamphibiouswetlandplants;heterophyllous,leavesformed under water by amphibious wetland plants;aquatic,leavesformedunderwaterbysubmergedaquaticplants(cf.Sculthorpe 1967).Ratesonamassbasis(AandB)wereconvertedto an area basis (C and D) using the published SLA data (Fig. 2, Appendix 1). The box– whisker plot shows the median, 10 and 90 per-centiles,minimumandmaximumvalues,andmeansareshownas'+';thedotintheterrestrialcolumnindicatesanoutlier.Differences amongstmeansof thefourplantgroups withineachpanel weretestedbyone-wayanalysis ofvarianceandTukey'smultiplecomparison tests.***P*ˏ0.01and****P*ˏ0.001.Meanswiththesameletterwithineachpaneldonotdiffersignificantlyatthe95%confidence interval.

stream waters are commonly supersaturated with $CO_2$,allowing even some terrestrial species to have adequate$P_N$forgrowthwhensubmergedinthesehabitats.

Thehigher$P_N$by aquatic leaftypesper unit masswithnear-ambient$CO_2$concentrations(~90 – 400 mM)demonstrates the higher C-return per unit of dry massinvestment by these leaf types in an underwater envir-onmentascomparedwithterrestrialtypes(Fig.1A).When external $CO_2$ was supplied at an elevated levelof~800 mM(Fig.1B),underwater$P_N$valuesby theaquaticandheterophyllousamphibiousleavesstillexceededthoseoftheterrestrialandhomophyllous leaf types. The low rates by terrestrial leaves evenwithelevated$CO_2$furtherdemonstratethelargediffusion limitations for $CO_2$ entry that restrict under-water$P_N$.

Expression of underwater $P_N$ rates on a surface areabasis, the units typically used in terrestrial plant physi-ology (whereas in aquatic sciences, rates are typicallyexpressed per unit dry mass), interestingly, removes dif-ferences between the terrestrial and aquatic leaf types,at both ambient and elevated $CO_2$ (Fig. 1C and D). TheorderofmagnitudeofhigherSLA(Fig.2)ofaquaticandmanyamphibiousleaftypesclearlysetsanupper

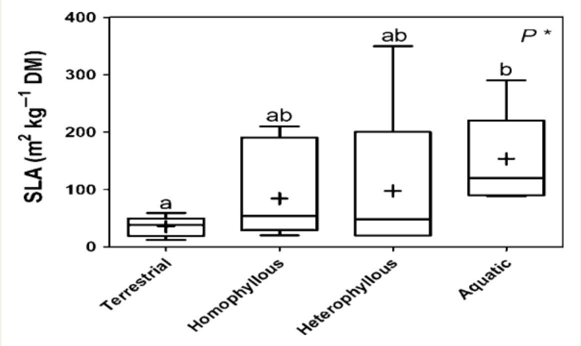

Fig. 2 Specific leaf area in terrestrial wetland plants, in amphibious homophyllous or heterophyllous wetland plants,and in submerged aquatic plants. The box-whisker maximumvalues,andmeansareshownas'+'.Speciesand data sources are listed in Appendix 1. Differences amongstmeans of SLA of the four plant groups were tested byone-wayanalysisofvarianceandTukey'smultiplecomparisontests. **

limit for $P_N$ on an area basis. Maximum $P_N$, however,would rarely be achieved in most aquatic environmentsowing to light and $CO_2$ limitations (Sand-Jensen 1989;Kirk 1994) so that the lower $CO_2$-saturated rates of $P_N$on an area basis for aquatic leaves would be of littleconsequencefortheirlifeunderwater.

Comparisons of the rates of underwater $P_N$ by terrestrialwetlandplant leaftypeswith thoseachievedbyaquatic leaf types are informative with respect to performancewhensubmerged(Fig.1),butherewealsoconsiderhowtheseratesunderwatercompareagainstthoseinair.FortheterrestrialwetlandspeciesinFig.1,wecouldonly find data on $P_N$ in air for three (*Carex elata*, *Ranuncu-lus repens* and *Phragmites australis*; Appendix 1); $P_N$ in airatambient$CO_2$was12.5–17mmolm$^{22}$s$^{21}$.Whensubmerged with $CO_2$ at levels near ambient (but well aboveair equilibrium in these habitats), the mean $P_N$ underwaterwasonly9%ofthatinair(cf.Fig.1C).Thus,underwater$P_N$isgreatlyreducedwhenterrestrialwetlandspeciesbecomesubmerged.

The analyses presented above for underwater $P_N$ byleaves of terrestrial wetland plants involved experimentsin which leaves growing in air were tested under water.Several terrestrial wetland species produce new leaveswhen submerged, and these can display some acclimationtotheunderwaterenvironment(e.g.thinnercuticles and thinner leaves; Mommer *et al*. 2007). Acclimatedleaves have decreased resistances against $CO_2$ and $O_2$movement across the cuticle and epidermis (MommerandVisser2005;Mommer*etal*.2007).Thebestexample is the several-fold reduction in cuticle resist-ance and thus the 69-fold higher underwater $P_N$ at anexternal $CO_2$ concentration of 250 mM by *Rumex palustris*(Mommer *et al*. 2006). Although a study of seven terrestrialwetlandspeciesestablishedtheformationofathinnercuticleasacommonresponsewhensubmerged,and demonstrated enhanced underwater gas exchange,the degree of this response was not correlated with submergencetolerance among these species (Mommer*et al*. 2007). These anatomical, and in some cases morphological (e.g. *R. palustris* leaves are also more elongated),changesinsubmergedleavesofterrestrialspecies are much more subtle than the altered leaf developmentdisplayedbyamphibiousheterophyllousspecies whichproducetrueaquaticleaftypeswhenunderwater(Nielsen1993).

Insummary,$P_N$byterrestrialwetlandplantsisreduced markedly when they are submerged. Leaves ofterrestrial wetland plants generally lack the numerousbeneficial leaf traits for underwater $P_N$ possessed byaquatic plants, although new leaves can display someacclimation (e.g. thinner cuticles and higher SLA). Inaddition, as discussed in the next section, some leavesof terrestrial wetland species retain a gas film when submerged,atraitthatalsoenhancesunderwater$P_N$.

## Leaf gas films enhance the net photosynthesisofsubmergedterrestrialwetlandplants

Many terrestrial wetland plants have water-repellent (i.e.hydrophobic) leaf surfaces, resulting in self-cleaning bywater droplets as these run off leaves (Neinhuis andBarthlott1997).Leafwaterrepellencehasbeenassessedbymeasurementofwaterdropletcontactangles with the surface (Adam 1963; Brewer and Smith1997; Neinhuis and Barthlott 1997)—angles of 1408ormore indicate a hydrophobic surface whereas angles of1108or less indicate a wettable surface. Water repel-lence (i.e. surface hydrophobicity) is determined by themicro- and nano-structures of the surface, as well aswax crystals (Wagner *et al*. 2003; Bhushan and Jung2006).

Superhydrophobic leaves retain a microlayer of gaswhen submerged, referred to as 'gas envelopes' (Setter*etal*.1989)and/or'leafgasfilms'(ColmerandPedersen2008). We prefer the term 'gas film' because althoughleaves of some species retain a gas layer on both sides(i.e. enveloped in gas), others retain a gas layer on onlyone side due to differences in hydrophobicity betweenadaxialandabaxialsurfaces(ColmerandPedersen

2008;Winkel*etal*.2011).Gasfilmsonleaveshavebeenobservedinfieldsituationsfor several terrestrialwetland species when submerged in lakes, ponds, riveredges and rice fields on floodplains: rice (Setter *et al*.1987); *P. australis*, cover of *New Phytologist*, Volume177(4); *Spartina anglica* (Winkel *et al*. 2011); and ownobservations(A.Winkel,T.D.ColmerandO.Pedersen).Information on the persistence of gas films on leaves withtimefollowingsubmergenceisscant;gasfilmsremained for at least 2 weeks (i.e. evaluation was terminated at 2weeks) on leaves of *Phalaris arundinacea*, *P. australis*and *Typha latifolia* (all with gas films on both sides) and*Glyceria maxima* (gas film on only the adaxial side) in acontrolled environment(Colmer and Pedersen 2008),but for some other species gas films diminish within afew days (own unpublished data; A. Winkel, T. D.ColmerandO.Pedersen).

GasfilmsonsubmergedleavesenhanceCO₂fixation,as first demonstrated for rice (9- to 10-fold increase;Raskin and Kende 1983). The beneficial effect of leafgas films to underwater $P_N$ has also been shown forotherterrestrial wetland species;at50mMdissolved $CO_2$gasfilmsincreasedunderwater$P_N$ by1.5-to 6-foldinleavesoffourwetlandspecies(ColmerandPedersen 2008). Data demonstrating the beneficial effect ofleaf gas films on underwater $P_N$ are shown for severalspecies in Fig. 3. Apparent resistance to $CO_2$ entry, atenvironmentally relevant $CO_2$ concentrations in the submergence water, was ~5-fold less in leaves with gasfilms compared with those with gas films removed(riceand*P.australis*;Pedersen*etal*.2009).

Leaf gas films provide an enlarged gas– water interfacetopromotegasexchangewiththesurroundingfloodwater ($CO_2$ uptake during light periods; $O_2$ uptakeduringdarkperiods)(ColmerandPedersen2008;Pedersen*etal*.2009).In addition to the enlargedgas–water interface, leaf gas films might also enablestomatatoremainopenwhenleavesaresubmerged. By contrast, for leaves without gas films, stomata arehypothesizedtocloseuponsubmergence (Mommerand Visser 2005), so that $CO_2$ and $O_2$ must then transverse the cuticle (Mommer *et al*. 2004). The beneficialeffect of leaf gas films on underwater $P_N$ was not onlydemonstratedbythemarkeddecreaseswhenthesewere removed (Fig. 3), but also leaves with this featurehadhigherratesofunderwater$P_N$than leavesfromspecies without leaf gas films (Fig. 3). Thus, leaf gasfilmsappeartoenableratesofunderwater$P_N$byterrestrialleavessimilartothoseachievedbysubmergence-acclimatedleavesofterrestrialwetlandplants (data and discussion in Colmer and

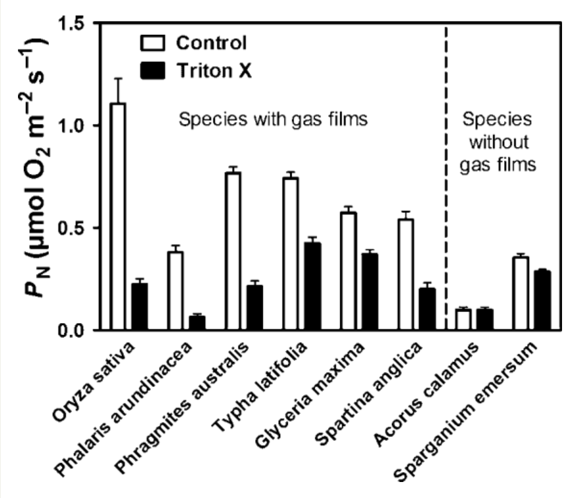

Fig.3Underwaternetphotosynthesisinterrestrialwetland plants with or without leaf gas films and when gas filmswere removed.Measurementsforsixspecieswere conducted with50mM$CO_2$at20 8Candphotosyntheticallyactiveradiation(PAR) of600mmolm$^{22}$s$^{21}$;theexceptionswere*Oryzasativa*(30 8C;PAR350mmolm$^{22}$s$^{21}$) and*S.anglica*(15mM $CO_2$;PAR550mmolm$^{22}$s$^{21}$).Thesereflectthehighertemperaturesintropicalricefields(*O.sativa*)andthelower$CO_2$inseawaterthatsubmerges*Spartina*marshes.Gas filmswereremovedfromleafsurfacesbybrushingwith0.05%Triton reduction in $P_N$. Data from Colmer and Pedersen (2008), Pedersen*etal*.(2009)andWinkel*etal*.(2011).

eafgasfil swouldb

shorttomediumperiodsofsubmergence,dependingonpersistence ofthefilms.Bycontrast,forspecieslacking leaf gas films but that produce new acclimatedleaves under water, these new leaves take several daysto produce so that PN would likely be less during theinitialsubmergence period, but continued productionofacclimatedleaveswouldbenefit these speciesduringmediumtoprolongedsubmergence.

Detailed knowledge on leaf gas films is available onlyforrice(onecultivaronly;Pedersenetal.2009).Measurements using O2 microelectrode profiling determinedthatgas filmthicknessvariedfrom,10to140 mm;positionaldifferencesmainlyresultedfromridges on leaves (i.e. gas films thinner at the tops ofridges, thicker between adjacent ridges). Using a 'buoyancy method' to measure gas volumes on the surfaces,andwithin,submergedleaves,showedthattissueporositywas19%(v/v)andthegasvolumeofthefilmswas 3.8timesmorethanthegaswithinthericeleaf.Diffusiveboundary layer widths adjacent to submerged leaveswith gas films were surprisingly larger than those adjacent to submerged leaves without gas films, so the enlarged water– gas interface provided by the gas filmswould have been the major mechanism that reducedresistance to gas exchange of the leaves when underwater. At dissolved $CO_2$ concentrations of relevance tofieldconditions(15 – 180 mM;e.g.inThailand,Setteret *al*. 1987; India, Ram *et al*. 1999), underwater $P_N$ wasenhanced 4- to 4.9-fold by gas films on leaves of rice(Pedersen *et al*. 2009). Underwater $P_N$ by leaves withgas films and $CO_2$ at near-ambient concentrations was22%of$P_N$inair.Whengasfilmswereremovedartificiallyfromleavesofcompletelysubmergedrice,tissuesugar levels and growth were both reduced. Thus, leafgasfilmscontributetosubmergencetoleranceofricebyenhancing$CO_2$entryforunderwater$P_N$.

TheexperimentsbyPedersen*etal*.(2009)alsoelucidated thatwhenriceleavesareinflowingwater(15mms$^{21}$;simulatinglowflowssuchasmightoccuracrossricefields),thegasfilmoscillatesandthetransition zone between mass flow in the bulk mediumanddiffusionintheboundarylayerwas wider, andmorevariable,thanforleaveswithoutagasfilm.Oscillations of leaf gasfilms in flowing water were also notedby Barthlott *et al*. (2010), and they reported that specialized surface hairs on the leaves of *Salvinia molesta* canstabilize the gas film, even in fast-flowing water (such asin streams). The leaf surface of *S. molesta* possesses‘eggbeater-shaped hairs’ that are hydrophobic exceptforthe tips,a feature that enablesgas film formationandretentionby‘pinning’thewater–airinterface(Barthlott *et al*. 2010). The presence of this feature wassuggested to prevent the formation and detachment ofbubbles that otherwise could occur when in fast-flowingwaters (Barthlott *et al*. 2010). This is a veryinterestingleafsurfacefeature,althoughtheecophysiologicalsignificance could be debated as *S. molesta* is a floatingplant not typically found in fast-flowing waters; the largegas volume trapped by these specialized structuresonthesurfaceoftheleaveswouldcontributesignificantlytothebuoyancyofthisfloatingplant.

Inadditiontoenhanced$CO_2$uptakeforphotosynthesis,leafgasfilmsalsoimprove$O_2$uptakeduringdarknessfromfloodwatersintoleaves(ColmerandPedersen2008; Pedersen *et al*. 2009). Thus, leaf gas films enhanceleaf$O_2$statusbothduringthedaytimeand duringnights,withbenefitsalsoofimprovedinternalaeration oftheentirebodyof submerged plants. Oxygenderivedfrom$P_N$duringlightperiods,as well as $O_2$entry from the floodwater into leaves when in darkness,moves internally via aerenchyma to roots of rice (Peder-sen *et al*. 2009) and rhizomes and roots of *S. anglica*(Winkel*etal*.2011).

Inconclusion,ourrecentstudiesofleafgasfilms(Colmer andPedersen2008;Pedersen*etal*.2009;Winkel

time after submergence and de-submergence.

*etal*. 2011)have supported the hypothesis by Setter*et al*. (1989), who observed this feature on submergedrice in field situations in Thailand, that gas films provide‘aninterfacebetweenthegasandwaterphasesforcollection of$CO_2$anddispersalof$O_2$duringthedayorcollectionof $O_2$ during the night’. This mechanism is analogous tothe gas layer (plastron) on some aquatic insects thatprovides an enlarged gas– water interface between thetracheary system and surrounding water (Thorpe andCrisp 1949; Raven 2008; Pedersen and Colmer 2012). Forterrestrialwetlandspecies,thefewdataavailableindicatethatleafgasfilmsenableratesofunderwater$P_N$similartothose achieved by submergence-acclimated leaves, inboth cases being higher than in terrestrial air-formedleaves without these features (data and discussion inColmerandPedersen2008).

## Conclusionsandfutureperspectives

Submergence can have adverse effects on terrestrialwetland plants because of restricted gas exchange andlow light. Floodwaters are variable in dissolved $O_2$, $CO_2$,light and temperature. Few data are available on keyenvironmentalparametersinvarioussubmergenceenvironments—yet these factors influence underwater$P_N$, plant growth and survival. Knowledge of floodwaterconditionswillenhanceone’sunderstandingofplantperformanceduringsubmergenceandenable thedesignofcontrolledexperimentsthatbettersimulateparticularsubmergenceenvironments.

Submergence tolerance of terrestrial wetland plants isinfluencedbyleaftraits.Althoughterrestrialwetlandplants generally lack the numerous beneficial leaf traitspossessed by aquatic plants, the few studies availabledemonstrate that some terrestrial species produce newleaveswith a thinner cuticle underwater and otherspossessleafgasfilms.Theimprovedgasdiffusionbetween leaves and floodwaters enhances underwater$P_N$andsocontributessignificantlytosugarand$O_2$supplyofsubmergedplants.However,studiesofleafgasfilm functioningareintheirinfancy.Ourprioritiesare
(i) quantification of the occurrence and persistence ofleafgasfilmsamongstawidenumberofwetlandspecies,anddeterminationofwhetherthistrait isrelatedtospeciesdistributionsinvariousflood-pronewetlands(cf.analysisofshootelongationtrait;Voesenek*etal*.2004) and (ii) evaluation of whether rice, or its relatives,possessesvariationinleafgasfilmformationandpersistence,andelucidationoftheunderlyinggeneticcontrolofthistraitusingthearrayofresourcesavailableinrice.

More broadly, there are surprisingly few studies on $P_N$by terrestrial wetland plants when emergent and whensubmerged.Alsolackingaremeasurementsof$P_N$with

Futurestudies should compare the performances of speciesfromvarioushabitats,usingarangeofappropriatebases of expression of $P_N$ rates (area, mass, chlorophyllandleafN)to facilitate interdisciplinary comparisonsbyaquaticandterrestrialplantbiologists.

## References

AdamNK. 1963. Principles of water repelency. In: Molliet J,ed.*Waterproofingandwaterrepellency*.Amsterdam: Elsevier.

AnderssonTN,LundegårdB.1999.Field horsetail(*Equisetum arvense*): effects of potasium under different light and nitrogenconditions.*WeedScience*47:47–54.

Armstrong W. 1979. Aeration in higher plants. *Advances in BotanicalResearch*7:225–332.

ArmstrongW,WrightEJ,LytheS,GaynardTJ.1985.Plantzonationandtheeffectsofthespring-neaptidalcycleonsoilaerationinaHumbersaltmarsh.*JournalofEcology*73:323–339.

Bailey-Serres J, Voesenek LACJ. 2008. Flooding stress: acclimationsandgeneticdiversity. *Annual Review of Plant Biology* 59:313–339.

Bailey-SerresJ,VoesenekLACJ.2010.Lifeinthebalance:asignal-ing network controlling survival of flooding. *Current Opinion inPlantBiology*13:489–494.

Baranenko VI, Falkovskii LN, Kirov VS, Kurnyk LN, Musienko AN,PiontkovskiiAI. 1990. Solubility ofoxygen and carbon dioxideinwater.*AtomicEnergy*68:342–346.Hamilton SK, Sippel SJ, Calheiros DF, Melack JM. 1997. An anoxiceventandotherbiogeochemicaleffectsof the Pantanalwetland on the Paraguay River. *Limnology and Oceanography*42:257–272.

Hellings SE, Gallagher JL. 1992. The effects of salinity and floodingon*Phragmites australis*. *Journal of Applied Ecology* 29:41–49.

Lassen C, Revsbech NP, Pedersen O. 1997. Macrophyte development and resuspension regulate the photosynthesis and productionofbenthicmicroalgae.*Hydrobiologia*350:1–11.

LenssenJPM,MentingFBJ,VanderPuttenWH.2003.Plantresponses to simultaneous stress of waterlogging and shade:amplified or hierarchiacal effects? *New Phytologist* 157:281–290.

Magnin NC, Cooley BA, Reiskind JB, Bowes G. 1997. Regulation andlocalization of key enzymes during the induction of Kranz-less,$C_4$-type photosynthesis in *Hydrilla verticillata*. *Plant Physiology*115:1681–1689.

MezianeD,ShipleyB.2001.Directandindirectrelationshipsbetween specific leaf area, leaf nitrogen and leaf gas exchange.Effects of irradiance and nutrient supply. *Annals of Botany* 88:915–927.

Millero FJ, Lee K, Roche M. 1998. Distribution of alkalinity in thesurfacewatersofthemajor oceans. *Marine Chemistry* 60:111–130.

MommerL,VisserEJW.2005.Underwaterphotosynthesisinflooded terrestrial plants: a matter of leaf plasticity. *Annals ofBotany*96:581–589.

MommerL,PedersenO,VisserEJW.2004.Acclimationofaterrestrialplanttosubmergencefacilitatesgas exchange underwater.*Plant,CellandEnvironment*27:1281–1287.

Mommer L, Pons TL, Visser EJW. 2006. Photosynthetic conse-quences of phenotypic plasticity in response to submergence:*Rumexpalustris*asacasestudy.*JournalofExperimentalBotany*57:283–290.

Mommer L, Wolters-Arts M, Andersen C, Visser EJW, Pedersen O.2007.Submergence-inducedleafacclimationinterrestrialspeciesvaryinginfloodingtolerance. *New Phytologist* 176:337–345.

Neinhuis C, Barthlott W. 1997. Characterization and distribution ofwater-repelent, self-cleaning plant surfaces. *Annals of Botany*79:667–677.

NielsenSL.1993.AcomparisonofaerialandsubmergedphotosynthesisinsomeDanishamphibiousplants.*AquaticBotany*45:27–40.

NielsenSL,NielsenHD.2006.Pigments,photosynthesisandphotoinhibition in two amphibious plants: consequences of varyingcarbonavailability.*NewPhytologist*170:311–319.

Nielsen SL, Sand-Jensen K. 1989. Regulation of photosyntheticrates of submerged rooted macrophytes. *Oecologia* 81:364–368.

Ogren WL. 1984. Photorespiration: pathways, regulation, and modification.*AnnualReviewofPlantPhysiology*35:415–442.

Parolin P. 2009. Submerged in darkness: adaptations to prolongedsubmergence by woody species of the Amazonian floodplains.*AnnalsofBotany*103:359–376.

PedersenO,ColmerTD.2012.Physicalgillspreventdrowningofmany wetland insects,spiders andplants.*Journal of ExperimentalBiology*,inpress.

PedersenO,Sand-JensenK.1992.Adaptationsofsubmerged*Lobeliadortmanna*toaeriallifeform—morphology,carbonsourcesandoxygendynamics.*Oikos*65:89–96.

PedersenO,Sand-JensenK,RevsbechNP.1995.DielpulsesofO$_2$and CO$_2$ in sandy lake sediments inhabited by *Lobelia dortmanna*.*Ecology*76:1536–1545.

Pedersen O, Vos H, Colmer TD. 2006. Oxygen dynamics duringsubmergenceinthehalophyticstemsucculent*Halosarciapergranulata*.*Plant,CellandEnvironment*29:1388–1399.

Pedersen O, Rich SM, Colmer TD. 2009. Surviving floods: leaf gasfilmsimprove$O_2$and$CO_2$ exchange, root aeration, andgrowth ofcompletely submergedrice.*ThePlantJournal* 58:147–156.

Pedersen O, Malik AI, Colmer TD. 2010. Submergence tolerance in*Hordeummarinum*:dissolved$CO_2$determinesunderwaterphotosynthesis and growth. *Functional Plant Biology* 37:524–531.

Pedersen O, Pulido C, Rich SM, Colmer TD. 2011*a*. *In situ* $O_2$ dynamics in submerged *Isoetes australis*: varied leaf gas permeabilityinfluencesunderwaterphotosynthesisandinternal$O_2$. *JournalofExperimentalBotany*62:4691–4700.

Pedersen O, Rich SM, Pulido C, Cawthray GR, Colmer TD. 2011*b*.Crassulacean acid metabolism enhances underwater photosynthesisanddiminishesphotorespirationintheaquaticplant*Isoetesaustralis*.*NewPhytologist*190:332–339.

Pérez-LlorénsJL,BrunFG,AndríaJ,VergaraJJ.2004.Seasonal and tidal variability of environmental carbon related physico-chemicalvariablesandinorganicCacquisitionin*Gracilariop*